1250-Plat
ATP Acts as Switch for Toggling Calreticulin Between its Lectin and Chaperone Function
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Calreticulin (CRT) is a lectin-binding chaperone that plays an important role in the assembly and folding of the major histocompatibility complex (MHC) class I proteins that present antigenic peptides on the cell surface and enable their recognition by T-cells. CRT binds to both specific oligosaccharide structures on MHC class I during their folding as well as a polypeptide part of MHC and thus plays a crucial role in stabilizing folding intermediates, preventing aggregation, and allowing the MHC protein to attain its native structure. Recent experiments have shown that the initial interaction between CRT and a monoglucosylated MHC protein is glycan-driven with glycan-independent interactions representing a second step in the chaperone cycle of CRT. Still, what factors trigger a switch from glycan-dependent to glycan-independent mode of interactions between CRT and its substrate is not well understood. Through computational investigations involving molecular dynamics simulations and structure based molecular docking, we show that ATP-binding serves as a switch for toggling CRT between two distinct modes of interactions. Specifically, the binding of ATP on CRT at the location that is distant from the glycan binding site leads to reduction in the affinity of CRT toward glycans and thus acts as a switch that induces a disengagement of a glycan from CRT, and induces exposure of hydrophobic regions on the surface of the globular domain allowing CRT to act as a chaperone through favoring interactions with the polypeptide component of the MHC. Further, employing community network analysis we have predicted residues that participate in allosteric signaling between ATP and glycan binding site of CRT. Modifying these residues has a large effect on the communication pathways in CRT consistent with experiments and support our model of ATP as a switch for CRT function.

1251-Plat
Allosteric Opening of the Polypeptide-Binding Site When an Hsp70 Binds ATP
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The 70 kD heat shock proteins (Hsp70s) are ubiquitous and highly conserved molecular chaperones essential for cellular protein folding and proteostasis. Each Hsp70 has two functional domains: a nucleotide-binding domain (NBD) that binds and hydrolyzes ATP, and a substrate-binding domain (SBD) that binds extended polypeptides. NBD and SBD interact little when in ADP; however, ATP binding allosterically couples the polypeptide- and ATP-binding sites. ATP binding promotes polypeptide release; polypeptide rebinding stimulates ATP hydrolysis. This allosteric coupling was poorly understood. To explore the molecular mechanism of this essential ATP-induced allosteric coupling, we solved a crystal structure of an intact Hsp70 from *E. coli* in complex with ATP at 1.96 Å resolution. NBD-ATP adopts a unique conformation, forming extensive interfaces with a radically changed SBD that has its α-helical lid displaced and the polypeptide-binding pocket of its β-subdomain flipped open. Our biochemical analysis inspired by this structure provides a long-sought mechanistic explanation of how ATP binding allosterically opens the polypeptide-binding site.

1252-Plat
Stability and Dynamics of Alpha Crystallin Oligomers Probed by FRET and FCS Reveal Persistent Oligomerization Under Dilute Conditions
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α-Crystallin is the major protein component of the human lens and plays an important role in the prevention of cataracts. α-Crystallin (αX) oligomers exist as a range of sizes varying from approximately 20-40 monomers. αX has two isoforms, αX-A and αX-B, which share high sequence similarity and define the common α-Crystallin fold found in many small heat shock proteins (sHSPs). αX-A and αX-B are hypothesized to play two important roles within the lens. First, they contribute significantly to the uniform, high protein density within the lens that enables it to cleanly focus light. Second, αX-A and αX-B both function as sHSPs that bind to misfolded proteins and prevent the formation of large, insoluble protein aggregates (the beginning of cataracts). Determining the mechanisms of αX chaperone function, oligomerization, and how the two are related will further the understanding of sHSPs in general and may lead to new preventions and treatments of cataracts. Several crystallographic studies of αX dimers have revealed a C-terminal strand exchange across a dimer interface. The dynamics of this C-terminus has been implicated in chaperone function and oligomer stability. Using fluorescence correlation spectroscopy (FCS) and intermolecular Förster resonance energy transfer (FRET) under both ensemble and single molecule conditions we investigate the stability of αX-B oligomers and their rates of subunit exchange. We present a method for disaggregating these high order oligomers under dilute conditions as well as describe conditions for generating kinetically trapped oligomers stable at nM concentrations.

1253-Plat
Enhanced Chaperone Clustering Facilitates Protein Folding in the Endoplasmic Reticulum of Yeast
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The chaperone BiP plays several roles in the endoplasmic reticulum (ER): translocation, protein folding, ER-associated degradation, and a modulating function in Ire1p-regulated ER stress. Experimental evidence has suggested the existence of BiP heterogeneity in the ER. A cooperative mechanism known as entropic pulling has been proposed to explain how the molecular interaction of multiple BiP molecules on unfolded proteins occurs. We have developed a model to explore the potential advantages of the binding of multiple BiP molecules in the facilitation of protein folding in the ER to explain the heterogeneity, and take advantage of entropic pulling.
Simulation scenarios were enacted to gauge the effectiveness of multiple chaperone binding in protein folding. Using two metrics: folding efficiency and chaperone cost, we determined that the single binding site model had a higher efficiency than multiple binding models, in the absence of cooperativity. Through entropic pulling, however, multiple chaperones do work in concert to facilitate the resolubilization and ultimate yield of folded proteins. Under a cooperative scenario, multiple binding models used fewer chaperones and enjoyed a higher folding efficiency than the single binding site model.
In conclusion, our in-silico experiments reveal that clusters of BiP molecules bound to unfolded proteins could enhance folding efficiency through cooperative action via entropic pulling.

Platform: Protein-Lipid Interactions II

1254-Plat
Molecular Mechanisms of High-Affinity Phosphoinositide Binding by the Tandem C2 Domains of Granuphilin/Slp-4
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Membrane-targeting proteins are crucial components of many cell signaling pathways, including the secretion of insulin. Granuphilin, also known as synaptotagmin-like protein 4, functions in tethering secretory vesicles to the plasma membrane prior to exocytosis. Granuphilin docks to insulin secretory vesicles through interaction of its N-terminal domain with vesicular Rab proteins; however, the mechanisms of its plasma membrane targeting and release are less clear. Granuphilin contains two C2 domains, C2A and C2B, that interact with the plasma membrane lipid phosphatidylinositol-(4,5)-bisphosphate [PI(4,5)P2]. Here we identify membrane-binding mechanisms, affinities, and kinetics of both granuphilin C2 domains using fluorescence spectroscopic techniques. Results indicate that both C2A and C2B bind anionic lipids in a Ca^{2+}-independent manner. The C2A domain binds liposomes containing a physiological mixture of lipids including 2% PI(4,5)P2 or PI(3,4,5)P3 with high affinity (apparent Kd of 2-5 nM), and binds nonspecifically with moderate affinity to anionic liposomes lacking phosphatidylinositol phosphate (PIPx) lipids. The C2B domain binds with sub-micromolar affinity to liposomes containing PI(4,5)P2 but does not have a measurable affinity for background anionic lipids. Both domains can be competed away from their target lipids by the soluble PIPx analogue inositol-(1,2,3,4,5,6)-hexakisphosphate (IP6), which is a positive regulator of insulin secretion. The results suggest potentially significant roles of granuphilin C2 domain-lipid interactions in the membrane docking and release of this protein during secretory signaling.

1255-Plat
NMR of Conditional Peripheral Membrane Proteins
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Conditional membrane proteins associate with membranes in response to binding specific ligands. We report the application of NMR techniques to gain insight into the structure, dynamics, and protein-membrane interactions of two fundamentally different conditional membrane modules: C1 and C2 domains, both